ATTACHMENT 9: MAJOR MILESTONES AND PROPOSAL TOPICS

1. MAJOR MILESTONES

SCHEDULE FOR CHEMICAL AND BIOLOGICAL TECHNOLOGIES DEPARTMENT NEW INITIATIVES FY2014-2016 PROGRAM – Amendment #3	
DATE	**EVENT**
September 28, 2015	BAA announced in FedBizOpps website
September 28, 2015	Begin registration at the DTRA proposal submission website
September 28, 2015	DTRA proposal submission website opens for receipt of Quad Chart/White Paper
November 3, 2015	Deadline to submit questions
November 13, 2015	Questions and Answers posted at FedBizOpps
November 30, 2015 No Later than 2:00 p.m. ET	Phase I proposal receipt deadline (Quad Chart/White Paper)
January 11, 2016	Phase II proposals invited
February 12, 2016 No Later than 2:00 p.m. ET	Phase II proposal receipt deadline
On or About April 7, 2016	Notification of selections and non-selections will be sent to Offerors
On or About September 15, 2016	Estimated First Award Date ("on or about" is used since this is an estimate)
Awards expected to begin approx. 150 days following initiation of negotiations [1, 2]	

Notes:

1. Actual award dates will vary based on receipt of funding, complexity, statutory requirements, quality of proposal, pricing considerations, DCAA audits of proposed rates, type of instrument, number of awards, and other considerations. All dates are subject to change.

2. Awards will be made subject to the availability of funds. All Offerors will be invited to begin negotiations upon notification of intent to award, and awards will be made as funds are available.

DIAGNOSTICS, DETECTION, and DISEASE SURVEILLANCE DIVISON (CBA)

Topic: CBA-01
In-Situ Protein and Gene Expression Platform Technologies for Host Response Biomarker Methods and Analysis

Goal: DTRA/CBA continues its efforts to characterize and develop multiplex biomarker (BM) panels on classifiers of human (and animal model) early exposure to biological and emerging threat agents. Proposals are now sought for the development of an integrated diagnostic prototype platform based on enhancements of methodologies for *in situ* single or sub-population cell-based analysis of differential transcriptomic, regulatory, and proteomic expression methods for verification and validation of these biomarker panels.

Background: Accurate measurement of the amount of a given biomolecule a cell produces is important for understanding molecular processes of cells. Bimolecular expressions within cells are a much regulated process whereby a single biomolecule can initiate a series of molecular networks that can lead to a disease. Understanding and taking advantage of how these expressions affects cellular phenotype at early stages of a disease can assist a diagnostician with critical information; for example, triggering reflexive or confirmatory testing, administrating aggressive treatments, initiating quarantine, and with triage risk stratification.
Current methods to measure biomolecules levels have remained relatively the same over the last few years and have shown to be unreliable and inaccurate. In addition, many technologies in use do not monitor changes in individual cells *in situ*. For this reason, an integrated approach is required in order to address limitations of standard bulk biomarker discovery approaches. This includes confounding protein and gene expression patterns in bulk measurements resultant from cell population (and sub-population) heterogeneity in complex biological systems and host responses in disease processes. Additional limitation includes loss of phenotypic context and capturing non-secreted and post-translational modified forms of biomarkers.
Objective: This topic seeks the delivery of an integrated diagnostic prototype platform based on advancements in *in situ* single or sub-population cell-based analysis. Recently, significant advancements have been made in enabling integrated technologies and methods/tools for *in situ* BM that can contribute to the development of a prototype platform. Examples of areas with recent technological and scientific advancements include *in situ* signal and target amplification, multiplexing/bar-coding, proximity-limited and digital *in situ* assays, recombinant small molecular weight ligand affinity binding reagents, novel cell separation (including microfluidic and micro droplet) approaches, identification of host molecular mechanisms, perturbed pathway networks, predictive candidates of differential classifier signatures, and improved image analysis at single molecule detection levels (including enhanced spatial and temporal resolution).

I. Proposals seeking the development of novel or improved approaches based on recent advancements in *in situ* single or sub-population cell-based analysis that will yield the delivery of an integrated diagnostic prototype platform are highly encouraged. Integrated prototypes should demonstrate and characterize temporal and intracellular host cell responses to infectious challenges. To accomplish this, the prototype should be verified with exposure studies; in vivo (animal) or ex vivo spiked blood samples demonstrated with relevant

bacterial and viral pathogen surrogates. Demonstrations with biothreat pathogens are NOT required. Furthermore, proposals should comprehensively address the following areas:

1. Development/application of methods enabling multiplex analyses of sixteen (16) or more biomarkers simultaneously. Biomarker expression methods should be demonstrated with:

    a) Protein expression signatures: measurement of protein abundance in single cells (or sub-populations)

    b) mRNA expression signatures: measurement of up- and down-regulated genes in single cells (or sub-populations)

        - Additionally, regulatory moieties including miRNA (and other small RNAs) expression and gene duplication/copy number variants will be deemed favorably

    c) Concurrent identification of heterogeneous cell sub-classes/states

2. Improved detection sensitivity and selectivity of *in situ* chemistries with unbiased amplification and absolute quantification. This may be accomplished by, but not limited to, one or more of the following methods

    a) *In situ* signal amplification

    b) *In situ* target amplification

    c) Enhanced *in situ* binding kinetics

3. Enhanced target cell permeability

    a) Live cell *in situ* analysis (i.e., without cellular lysis, fixation or biochemical perturbation of cells) are preferentially sought

4. Improved cell separation methods compatible with integration into a portable form-factor prototype

5. Miniaturization of optical/image analysis hardware, enhanced image analysis algorithms/software/analytics and integration into a portable form-factor prototype.

II. Offerors are encouraged to collaborate with other organizations in the Government, academia, and the private sector to broaden and strengthen their knowledge, experience, and capabilities.

Topic: CBA-02
Ultra-Rapid, Low Power Multiplexed PCR-based Molecular Diagnostics Point-of-Care Devices

The Ebola viruses (EBOV) outbreak in West Africa as well as other global Emerging Infectious Disease outbreaks highlights the need for ultra-rapid yet highly sensitive detection of bio- and emerging threat agents and disease diagnostic systems. Studies of the recent Ebola outbreaks indicate that use of rapid point-of-care diagnostic tests could significantly improve containment and treatment outcomes. Testing for subclinical infections (early cases) and late convalescent phase of many of these disease can prove challenging, considering the low pathogen loads of in the blood at these time points in the diagnostic window. Both high analytical sensitivity and assay stringency are both required to avoid BOTH false negatives and positives (i.e. to provide high Negative Predictive Values (NPV) and high Positive Predictive Values (PPV), with disease prevalence rates considered).

While Polymerase Chain Reaction (PCR) has demonstrated superior performance for molecular diagnostics, critical technology gaps in ultra-rapid thermal cycling, high power requirements limiting battery life, and elaborate sample preparation have prevented development of truly rapid and small form factor (i.e. hand-held or highly portable) devices for use in resource-limited and austere environmental settings. Recent research (1,2) has demonstrated significant enabling technologies that can address these issues. While previous efforts have attempted to reduce PCR times to ≤ 5 minutes, these approaches have generally led to compromises in amplification efficiency, amplicon yield, and target/multiplex capacity.

This topic seeks proposals to develop novel platform and method technologies that will fundamentally deliver a prototype device and multiplex assays utilizing ultra-rapid PCR cycling and low power requirements while maintaining desirable operational suitability characteristics. Successful candidates will demonstrate these capabilities by adapting prototypical bio- or emerging threat pathogen signature assays for at least one each Gram positive bacteria, Gram negative bacteria, RNA viruses and DNA viruses from NIAID Category A, B, and C Priority Pathogens list (http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/pages/cata.aspx) or CDC Bioterrorism Agents/Diseases list (http://emergency.cdc.gov/agent/agentlist.asp). While synthetic oligonucleotides may be used for initial assay development, purified nucleic acid and/or inactivated pathogens should be used for verification testing. Furthermore, novel approaches may be needed to incorporate multiplexed testing of at least 8 simultaneous analytes. Additionally, feasibility of integration of sample preparation and nucleic acid purification will receive high consideration as will ability to demonstrate above tests in a prototype "syndromic febrile panel" to provide differential diagnosis and utility across a broad range of etiological agents (i.e., intracellular organisms, parasites, etc.), diseases and clinical sample types and to provide information to support force health protection decision making.

The degree of innovation and responsiveness will be measured by the offeror's approach and ability to address the following attributes: achieve a high sensitivity and specificity as high as current state-of-art PCR-based tests/panels and utility with a broad range of disease and sample types while retaining operationally desirable characteristics:

a. Single sample cycling ≤ 5 minutes,
b. Time to result ≤ 10 minutes,
c. At least 5 fold improvement in power consumption from current state-of-art thermal cyclers/PCR instruments;
d. Ease-of-use, CLIA waiver-capable;
e. System weight with consumables for 40 tests less than 8 lbs.;
f. Performance under austere environmental conditions (at least 40°C/75% Relative Humidity); and

g. Integrated wireless communication for device-to-cloud result transmission will be considered a positive attribute.

Topic: CBA-03
Pre-Analytical Method Refinement: Novel Bio-Sample Collection, Preservation, and Preparation

Proposals are sought for pre-analytical method refinement by developing novel clinical sample collection, transport, preservation, and preparation methods for viral and bacterial biological and emerging threat pathogen diagnostic testing. Comprehensive solutions are sought in either or both of the following topics:

1. Safe, reproducible, and painless sample collection and transport devices. In a biothreat infectious disease agent outbreak, the high morbidity and mortality nature of the pathogen and the potential for transmissibility from infected bodily fluids (e.g., blood) presents a concern for the safety of both phlebotomists and laboratory testing personnel. Collection of whole blood by venipuncture or finger stick requires a trained phlebotomist and entails a risk of accidental needle stick. Also, in case of hemorrhagic disease sequelae, the venipuncture or lancet finger-stick site may present an ongoing risk for exposure and infectious waste bleed even with application of pressure dressing. Novel whole blood collection devices are sought with the following attributes:
    a. Safety-oriented device designs;
    b. Result in relatively pain-free use;
    c. Deliver standardized sample volumes (Range: ≤ 10-500 µL ± 10%);
    d. Delivered into appropriate "universal" transport media formulation (DNA, RNA, protein stabilization);
    e. Stable under austere environmental conditions (at least 40°C/75% Relative Humidity); and
    f. Capability of mating directly with Point of Care cartridge or paper diagnostic device will be considered a positive attribute.

2. Novel biomolecule stabilization materials/methods to preserve pathogen and host analytes from whole blood and swab samples, to include:
    a. Intact pathogen (i.e., surface antigens);
    b. Shed/secreted proteins/toxins;
    c. Human host immune cells and protein and metabolic biomarkers; and
    d. Pathogen and host nucleic acids (compatible with molecular methods including target amplification and sequencing)

Analytes of interest for stabilization would ideally be from pathogens on the NIAID Category A, B, and C Priority Pathogens list (http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/pages/cata.aspx) or the CDC Bioterrorism Agents/Diseases list (http://emergency.cdc.gov/agent/agentlist.asp). Preservation materials/methods should support analysis across multiple analytical platforms. Formulations must be compatible with high priority clinical specimen types (i.e., whole blood

and relevant cellular and extracellular components; sputum and nasal swabs; urine; fecal material) as well as recovered tissue samples. Commercial-off-the-shelf general excipients (e.g., trehalose) are NOT deemed responsive. Samples must be stabilized without cold chain and without losing pathogen viability (e.g., for culture confirmation). Verification of method performance characteristics is desired in relevant animal models and spiking studies. Collaborations with BSL-certified laboratories are encouraged, although simulants can be substituted if viability capability is claimed. Methods/formulations that modify pathogen infectivity, antimicrobial resistance, etc. (i.e., Dual Use Research of Concern) will NOT be judged responsive.

Topic: CBA-04
Discovery of Proteomic Signatures to Distinguish Pathogen Growth Conditions

Microbial pathogens can occur naturally in the environment and may cause human disease through inadvertent exposure with environmental or animal hosts. For example, plague is an acute infectious zoonotic disease caused by the highly virulent bacteria *Yersinia pestis*. Plague infections in humans typically result from the bites of fleas that feed on infected rodents, although person-to-person transmission can occur if the bacteria are inhaled. Specific and sensitive diagnostic tools are available to identify the causative pathogen of these infectious diseases through genomic analysis. Recent research has demonstrated that for bacteria grown in laboratory media, residual peptides from the medium can be identified along with peptides from washed bacteria (1). This suggests that proteomic signatures of laboratory culture methods could be beneficial to distinguish natural (wild) from laboratory-grown microbial organisms. To assist with identifying the source of clinical infection, research is needed to understand whether proteomic signatures that distinguish natural and laboratory-grown organisms remain after infection of an animal host.

This topic seeks proposals using proteomic analysis to discover signatures to determine whether an infection was caused by naturally-occurring or laboratory-grown microbial organisms. Proteomic methods are sought for identification of microbial proteins and toxins. Successful candidates will discover signatures for at least one Gram positive bacterium, Gram negative bacterium, RNA virus, or DNA virus from NIAID Category A, B, and C Priority Pathogens list (http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/pages/cata.aspx) or CDC Bioterrorism Agents/Diseases list (http://emergency.cdc.gov/agent/agentlist.asp). Proposers should provide supporting data, or an approach to generate data, on protein markers that are differentially expressed in strains from natural environments versus laboratory cultures as reference data for investigating proteomic profiles of host/animal-passaged virulent strains. Verification of signatures is desired in relevant animal models. Collaborations with BSL-certified laboratories are encouraged.

References

Clowers BH1, Wunschel DS, Kreuzer HW, Engelmann HE, Valentine N, Wahl KL. Characterization of residual medium peptides from Yersinia pestis cultures. Anal Chem. 2013 Apr 16;85(8):3933-9. doi: 10.1021/ac3034272. Epub 2013 Apr 3.

Topic: CBA-05
Personal Chemical Hazard Detector

Proposals are sought for novel technologies to provide low-cost, rapid-response, lightweight detectors for chemical threats in the environment. There are currently shortfalls in the ability to provide real-time detection of airborne chemical threats in order to inform force protection actions. Existing approaches for tactical detection do not provide sufficient sensitivity with an acceptable false alarm rate in a person-portable or wearable form factor.

Technical approaches sought will be applicable to the detection of airborne chemical threats in the environment, to include chemical warfare agents (CWAs) and toxic industrial chemicals (TICs). The overall approach should at a minimum provide initial detection or classification at 10 minute Critical (optionally to 10 minute Negligible) Military Exposure Guideline (MEG)[1] concentrations, with sufficient discrimination against background interferents, in order to indicate a hazardous environment. The sensor is expected to be tactical/person-wearable in size, weight, and power with low logistical burden. Capabilities that can also provide indication of O_2 and flammable gas concentrations at or above the lower explosive limit (LEL), are of interest, but at a lower priority level.

Proposed solutions should currently be at Technology Readiness Level (TRL) 3 or above, and representative data demonstrating current performance must be included. Detection systems should include all required components, including batteries appropriate for extended mission lifetime. Proposals should detail the planned system components and should include specifications for all components and expected operational parameters. Proposals must specify the analytes to be measured, as well as the current and anticipated detection limits, response times, and dynamic ranges. Proposals should also include any data relevant to specificity/false alarm rate/detection of analytes in complex environmental backgrounds, as well as projected system false alarm rate. Solutions based on non-specific detection of air composition change over background levels without further classification will not be considered. Responses must include current metrics and projected estimates of size, weight, and power requirements, as well as detailing of expected operational lifetime and instrument costs. Projected size, weight and power as well as performance parameters should be compared with the desired parameters listed in the specifications below.

Required Capabilities:

- Detection, with alarm or other rapid indication, of CWA and TIC chemical vapors at 10-minute Critical MEG concentrations
- Identification by chemical class of nerve, blister, or TIC type:
    - Nerve and blister chemical vapors of chemical purities greater than 40%. Analytes of interest include GA, GB, GD, GF, HD, HN3, Lewisite (required) and VX (optional)
    - TIC chemical vapors of chemical purities greater than 80%. Analytes of interest include, but are not limited to,hydrogen cyanide (HCN), cyanogen chloride (ClCN), phosgene ($COCl_2$), Cl_2, H_2S, NH_3, NO_2, and SO_2
- Response time of 30 seconds to 10-min Critical MEG levels

Optional Capabilities:

- Detection of CWAs and TICs in less than 180 seconds at 10-min Negligible MEG levels
- Indicate O_2 concentrations below 19.5% (O_2 deficient) and/or above 23.5% (O_2 enriched)
- Indicate flammable gas concentrations at or above the lower explosive limit (LEL)
- Detection of chemical threats in aerosol form

Device Specification Goals:

- Less than 325 cm^3 volume and 0.5 kg weight
- Operation on standard off the shelf commercial batteries for 8 continuous hours between -10°C and 35°C and atmospheric water vapor content (WVC) between 5-30 g/m^3.
- False alarm rate of less than one per 72 hours, with a stretch goal of less than one per week

Offerors should plan for a 1 year base period of performance, with up to two 1-year option periods, Sufficient progress toward the topic objectives should be made in order to support a go/no-go decision prior to the end of each year of effort.

References:

1. TG-230 Environmental Health Risk Assessment and Chemical Exposure Guidelines for Deployed Military Personnel: http://phc.amedd.army.mil/PHC%20Resource%20Library/TG230.pdf

Topic: CBA-06
New Analytics and Data Sources to Support Global DoD Biosurveillance

Background: The Biosurveillance Ecosystem (BSVE) is a collaborative platform for analysts to fuse and analyze datasets related to infectious diseases into actionable decision making products. The BSVE is a cloud-based system on the Amazon Web Services (AWS) cloud allowing spontaneous scaling for data storage and computational needs. The BSVE is developed using an open source software architecture allowing easier integration, increased transparency for a broader user base and customizability, and reduced costs due to licensing. The BSVE ingests and utilizes large data streams such as open source social media feeds, RSS feeds, disease ontologies, de-identified diagnostic results, historic outbreak data, zoonotic data, and non-health data as well as machine learning and natural language processing algorithms to intelligently identify aberrations in disease signals.

Objective: Ensuring state of the art technologies are made rapidly accessible through the BSVE, this topic seeks to develop analytic applications (apps) to synthesize and interrogate multiple sources of data to provide high confidence in the prediction and early warning of disease events. Metrics shall be devised such that successful utilization of these analytic tools will result in a measureable impact on the bioevent timeline.
Research areas of interest, which are not ranked in order of priority, include:

- Quantify the burden of disease or field risk based on event-based surveillance and historical baseline data.
- Utilize plant and/or animal disease to provide early prediction of possible disease emergence.
- Tools to support DoD personnel responding to a chemical or biological event by providing a wide range of information on the agent(s), including agent identification support, physical characteristics, human health information, and containment and suppression advice.

BSVE Compatibility: Analytics developed, and their associated data ingest requirements, should aim to be flexible, extensible, automated, and sustainable. Analytics should be developed to plug into the BSVE using the BSVE Software Developer's Kit (SDK).

Topic: CBA-07
Making Disease Forecasts Actionable: Novel displays, Uncertainty Quantification and Ensemble Approaches

Objective: As disease forecast modeling capabilities begin to increase there is a need to transform the outputs of these models into actionable decision making information. Applying statistical uncertainty and quantification can be used to develop a consensus of model predictions and provide a single result which includes standard uncertainty values. The ability to display multiple model results is necessary for analysts to accurately represent the realm of possibilities, likelihood and uncertainty.

Research areas of interest, which are not ranked in order of priority, include:

- Applications to incorporate multiple disease model results into a single view and provide a capability for analysts to seamlessly edit/modify existing forecasts.
- Methods and applications to develop statistical uncertainty estimates from multiple disease model outputs.
- Methods of constructing disease forecast model ensembles; both for forecasting, for example, disease season outbreak/intensity and disease transmission, and spread through a population.

Topic: CBA-08
Field Forward Diagnostics

Background: The Field Forward Diagnostics (FFDx) program is a collaborative effort focused on leveraging mature, minimally complex diagnostic devices ((buddy-care and Role 1) and testing in resource limited settings. Research involves assessing the ability of these devices to utilize multiplexed assays with high specificity/sensitivity to diagnose syndromically relevant pathogens. The devices are then integrated wirelessly to the DTRA-developed Biosurveillance Ecosystem (BSVE) to share data with biosurveillance analysts and to inform advanced analytic applications for decision making.

Objective: This topic area seeks to expand existing DTRA FFDx research, development and demonstration efforts at CONUS and OCONUS sites. Successful proposals will demonstrate the ability to coordinate research across multiple laboratories including the capability to develop research/testing protocols in coordination with proper institutional or Ministry of Health (MOH) channels, execute a time-sensitive (per local endemic diseases) sample collection program with confirmatory testing and the ability to uplink collected data to a cloud-based bioinformatics system (e.g., the DTRA-developed BSVE.)

Successful proposals will exhibit these key elements:

- Utilization of established clinical study locations to create protocols and obtain IRB (possibly MOH) approvals.
- Collection of a statistically significant minimum set of data based on experimental assay characteristics and local disease prevalence for DoD-relevant diseases as specified by DTRA. Initial focus should be on Lassa, chikungunya, dengue or *Yersinia pestis.*
- Ability to exploit DTRA-provided "for research use only" point of need (PON) assays using various collection matrices. Preference is to utilize whole blood samples.
- Ability to perform confirmatory testing for each disease set identified in the PON or make arrangements to ship samples to an external lab for completion.
- Ability to collaborate with DTRA partner agencies which may provide clinical management and oversight.

Topic: CBA-09
Predicting Disease (Re)Emergence

Objective: This topic seeks to improve the prediction of disease emergence and reemergence, including estimates of model uncertainty, using data and knowledge from current epidemics. Diseases of concern include both those with the potential to occur internationally as well as domestically. A current example of emergent and re-emergent diseases and potential areas of research is given below for illustrative purposes, but research on other diseases, regions, and sources of model uncertainty is welcome.

Dengue, chikungunya, and zika fever are emergent and re-emergent human diseases whose viral causative agents share the same mosquito vectors. In the Americas, successive and concurrent epidemics of first dengue, then chikungunya, and now zika fever are occurring. In addition to sharing the same mosquito vectors, all three diseases have similar symptoms in humans, making disease-specific clinical diagnosis unreliable in the absence of confirmatory laboratory testing. Health and surveillance systems in the affected tropical and subtropical regions of the Americas vary in their ability to specifically diagnose these diseases; the proportion of laboratory-confirmed cases for dengue and chikungunya, for example, ranges between 0 and approximately 30% of suspected cases reported to the Pan American Health Organization (PAHO), with both diseases being reported in many countries. With the recent emergence of zika fever in Brazil, the ability to estimate the relative incidence of each disease is decreasing. Accurate estimation methods that can be used independently of definitive laboratory diagnosis are needed to establish the incidence of each disease. These in turn will be used to predict disease spread and quantify model uncertainty.

Research questions and areas of interest, which are not ranked in order of priority, include:

- In areas in which two or more of the viruses are circulating, are there ecological or virus-specific constraints on one or more of the viruses, given that they are competing for the same hosts and vectors?
- Are there any differences in the ecology, epidemiology, symptomatology, or other indicator that can be measured, either indirectly or remotely, that improve the ability to differentiate between the three diseases?
- Can knowledge gleaned from the appearance and spread of the three successive diseases in the Americas or elsewhere be used synergistically to inform disease forecasting?
- Accurate disease forecasting models require quantified measures of uncertainty for each input. The uncertainty surrounding current incidence data is considerable. What de facto disease surveillance practices exist in each affected or at-risk country in the Americas? Contributors to uncertainty of incidence data, which vary by country, include:
  - The probability that a person with compatible disease symptoms will seek medical care.
  - Circumstances under which clinical specimens are collected and tested for each virus.
  - The national and subnational laboratory capacity for each virus.
  - How routinely and with what barriers the suspected and confirmed cases get reported to public health authorities.
  - Is zika fever a reportable disease in each country? If so, are individual cases reportable or only outbreaks?
  - The case definition used for each disease in each country.
  - Whether case definitions are applied consistently, and by persons qualified to do so.
  - What public health follow up is conducted to classify reported disease cases?
  - The time delay between case identification and case reporting to public health authorities, for both suspected and confirmed cases.

Topic: CBA-10
Evaluation of Wearable Technologies for Early Indication of Health Changes

Background: To ensure mission success, warfighters need to remain healthy. Recent advancements in wearable physiological monitoring technologies provide an opportunity to evaluate the utility of health data for early warning of exposure to a biological and/or chemical agent. Earlier warning of a possible infection or chemical exposure could allow for a more timely treatment regimen which may increase the ability of warfighters to perform their missions.

Objective: This topic seeks to inform research investments by evaluating existing commercial/government off-the-shelf (COTS/GOTS) physiological monitoring technologies

(e.g., biostamps, wearables, etc.) that can be "worn" by personnel to evaluate health status. Technologies that could be used to determine if personnel have indications of an infectious disease or exposure to chemical substances are of the highest priority. Successful proposals will demonstrate an agile evaluation methodology including plans to inform future research investments that optimally leverage advanced COTS/GOTS products as the architectural backbone.

Research questions and areas of interest:

- A cohort study evaluating the correlations between physiological monitoring data and health record information.
- Evaluation of the minimum frequency of physiological monitoring data needed for strong correlations to health effects.
- Ability to provide earlier warning of health incidents using physiological data.
- Comparisons to disease data sources such as google trends, social media information, and/or diagnostics results.
- Tools/Applications/Data Warehouse Standards to analyze the physiological monitoring data in near real-time from multiple users with integration into the Biosurveillance Ecosystem (BSVE), a collaborative platform for analysts to fuse and analyze datasets related to infectious diseases into actionable decision making products.
- Ability to download data from many individuals in an automated, centralized, and timely manner.

Topic: CBA-11
Can Social Media Predict the Future?

Background: Analysis and correlation of social media data such as tweets, blogs, and Facebook posts have shown to be useful in providing early indications of social unrest. It has been theorized that similar methods may be applied to these types of data to assist with disease predictions and forecasts. However, forecasting disease emergence and subsequent spread is complex and there are limited datasets available for verification of such forecasts.

Objective: The topic seeks proposals to conduct studies that will utilize well sampled datasets (for verification purposes) to assess the effectiveness of exploiting social media data to represent a current state and to predict the future. For the purpose of this study, proposals should investigate whether social media data can be analyzed/manipulated to determine the current and future state of the atmosphere; for example temperature, humidity, and/or precipitation, for a given geographical regime. Metrics comparing the predicted state versus actual records should be presented to illustrate the applicability of social media for prediction. All studies should be completed within one year and result in a report.

TRANSLATIONAL MEDICINE DIVISION (CBM)

Topic: CBM-01
Late Discovery and Development of Novel Therapeutic Approaches to Combat Antimicrobial Resistance in Biological Threat Agents

Objectives: This topic seeks milestone-driven proposals focused on the late discovery and development of novel antimicrobial therapies that have the potential to potently and specifically treat multiple drug resistant bacterial infections, including those caused by priority DoD bacterial threat agents (*F. tularensis, B. anthracis, Y. pestis,* and *C. burnetii* with appropriate multi-drug resistant (MDR) surrogates of unavailable MDR biothreat pathogens). Responsive approaches may circumvent AMR mechanisms or potentiate the therapeutic efficacy of existing antibiotics by targeting specific mechanisms of resistance. Development of combination therapies that might result in the re-sensitization of resistant bacteria to legacy antibiotics will also be considered. Less traditional (non-small molecule) therapeutic approaches and modalities are encouraged and those targeting the host, either by direct interdiction of infection or immunomodulation, are particularly sought.

Responsive proposals will include preliminary data for candidate products towards a defined Target Product Profile and regulatory path (both required at the Phase II proposal stage). Priority will be given to proposals that fulfill more advanced stages of development either previously, through work conducted in this proposal, or through conjunction of other complementary work outside this proposal. At minimum, responsive discovery efforts should be no less mature than lead validation and optimization (lead candidate must be identified).

The following is not of interest and considered outside of the scope of the topic:

1. Basic research studies focusing on host-pathogen interaction including target identification and/or validation or structural analysis of antibacterial targets.
2. Efforts in early discovery or focusing on high throughput screening.
3. Efforts focused on therapeutics for non-resistant strains of *F. tularensis, B. anthracis, Y. pestis,* and/or *C. burnetii* without a concurrent approach against MDR surrogates, or solely on non-BWA.
4. Efforts directed toward the reformulation of FDA approved, late-development, or failed therapeutic candidates for BWA or other indications.

Offerors are encouraged to develop R&D collaborations with other organizations in Government, academia, and the private sector to broaden and strengthen their capabilities. Where possible, Offerors are encouraged to take advantage of specialized resources in DoD and other Government agencies such as facilities/capabilities for biocontainment, collections of biothreat pathogens, Core testing, or advanced manufacturing.

Because collections of AMR and MDR BSL-3 biodefense pathogens are not currently available to the broad community, predicted efficacy for AMR and/or MDR biodefense pathogens may be demonstrated using clinical isolates of other pathogens with variable or high-level characterized resistance to specific antibiotics (i.e. Methicillin Resistant Staphylococcus aureus (MRSA), Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, etc.). However, these non-biothreat BSL-2 strains should only be used to assess the ability of a therapeutic, or combination of therapeutics, to overcome resistance mechanisms and effectively inhibit microbial growth, etc. Therefore, efforts should not focus on the development of antibiotics that are specific to these surrogate pathogens or their mechanisms of pathogenicity. Additionally, the government currently offers a Core testing capability to perform in vitro and/or in vivo screening of compounds (lead, advanced, or licensed) alone or in combination against an extensive panel of biodefense pathogens, as well as a panel of MDR ESKAPE pathogens, to generate MIC90 and/or murine survival data at no cost and with no intellectual property implications to the providing party. Respondents interested in acquiring additional information may inquire through the BAA. It should be noted that during the course of performance of proposals selected for funding, in vitro performance of promising candidates or combinations of candidates will be validated, at the cost of the government, by this Core testing capability per government use rights.
The DoD has awarded a contract (W911QY-13-C-0010) to establish an Advanced Development and Manufacturing capability (ADM). In addition to providing a BSL-3 capable, multiproduct manufacturing facility for biologic products, the ADM and a consortium of teaming partners can support development of medical countermeasures from discovery through FDA approval. This includes the facilities, equipment and expertise necessary to perform nonclinical, clinical, process development, and regulatory activities. Respondents interested in discussing potential collaborations with the ADM may inquire through the BAA.

Topic: CBM-02
Advanced Bacterial Antimicrobial and Anti-Infectives with Novel Mechanisms of Action

Objectives: This topic seeks milestone-driven proposals focused on the development of antimicrobial therapies that have the potential to potently and specifically treat multiple drug resistant bacterial infections, including those caused by priority DoD bacterial threat agents (B. pseudomallei, F. tularensis, B. anthracis, Y. pestis, and/or C. burnetii).
Responsive proposals will include candidates with supporting data for a unique, novel mechanism of action that does not overlap with marketed antibacterials for which drug resistant strains have been identified. An exception to this rule is a combination therapy approach that introduces a candidate with a novel MoA to enhance the utility of a currently marketed antibiotic against which significant resistance has arisen. Drugs targeting topoisomerase/gyrase will not be considered.
Priority will be given to proposals that fulfill more advanced stages of development either previously, through work conducted in this proposal, or through conjunction of other complementary work outside this proposal. Ideally, the candidate is broad-spectrum and should have, at minimum, in vivo proof of concept data against biothreat agents and commenced preclinical development toward IND filing within 2 years. Candidates that have initiated Phase I clinical studies for safety, tolerability and PK for clinical indications are of particular interest.

Responsive proposals will include preliminary data for candidate products toward a defined Target Product Profile and a regulatory plan (both required in a phase II proposal, if invited). The following are not of interest and considered outside of the scope of the topic:

1. Basic research studies focusing on host-pathogen interaction including target identification and/or validation or structural analysis of antibacterial targets.
2. Efforts focused on therapeutics for non-BWA strains solely or non-resistant strains of F. tularensis, B. anthracis, Y. pestis, and/or C. burnetii without a concurrent approach against MDR surrogates.
3. Efforts directed toward the reformulation of FDA approved, late-development, or failed therapeutic candidates for BWA or other indications.

Offerors are encouraged to develop R&D collaborations with other organizations in Government, academia, and the private sector to broaden and strengthen their capabilities. Where possible, Offerors are encouraged to take advantage of specialized resources in DoD and other Government agencies such as facilities/capabilities for biocontainment, collections of biothreat pathogens, Core testing, or advanced manufacturing.

Because collections of AMR and MDR BSL-3 biodefense pathogens are not currently available to the broad community, predicted efficacy for AMR and/or MDR biodefense pathogens may be demonstrated using clinical isolates of other pathogens with variable or high-level characterized resistance to specific antibiotics (i.e. Methicillin Resistant Staphylococcus aureus (MRSA), Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, etc.). However, these non-biothreat BSL-2 strains should only be used to assess the ability of a therapeutic, or combination of therapeutics, to overcome resistance mechanisms and effectively inhibit microbial growth, etc. Therefore, efforts should not focus on the development of antibiotics that are specific to these surrogate pathogens or their mechanisms of pathogenicity.

Additionally, the government currently offers a Core testing capability to perform in vitro and/or in vivo screening of compounds (lead, advanced, or licensed) alone or in combination against an extensive panel of biodefense pathogens, as well as a panel of MDR ESKAPE pathogens, to generate MIC90 and/or murine survival data at no cost and with no intellectual property implications to the providing party. Respondents interested in acquiring additional information may inquire through the BAA. It should be noted that during the course of performance of proposals selected for funding, in vitro performance of promising candidates or combinations of candidates will be validated, at the cost of the government, by this Core testing capability per government use rights.

The DoD has awarded a contract (W911QY-13-C-0010) to establish an Advanced Development and Manufacturing capability (ADM). In addition to providing a BSL-3 capable, multiproduct manufacturing facility for biologic products, the ADM and a consortium of teaming partners can support development of medical countermeasures from discovery through FDA approval. This includes the facilities, equipment and expertise necessary to perform nonclinical, clinical, process development, and regulatory activities. Respondents interested in discussing potential collaborations with the ADM may inquire through the BAA.

Topic: CBM-03
Novel Small Molecule Medical Countermeasures Development Targeting Filoviridae Pathogenesis and Resistance

Objectives: The objective of this topic is to solicit proposals for the development of novel and innovative small molecule therapeutics to prevent (post-exposure prophylaxis) or treat (post-exposure therapeutic) for one or both Filovirus family members (Marburgvirus and Ebolavirus), and any combination of the five Ebolavirus species: Taï Forest (formerly Ivory Coast, Sudan, Zaire, Reston or Bundibugyo)

Background: Ebola and Marburg hemorrhagic fevers (EHF and MHF) are caused by the Filoviridae family of viruses, Ebolavirus and Marburgvirus, respectively. These severe diseases have high mortality rates, approaching nearly 90% in humans. EHF and MHF are classified as select agents; World Health Organization Risk Group 4 Pathogens (requiring Biosafety Level 4-equivalent containment), National Institutes of Health/National Institute of Allergy and Infectious Diseases Category A Priority Pathogens, and Centers for Disease Control and Prevention Category A Bioterrorism Agents. Therefore, post-exposure measures against these viral hemorrhagic fevers are a high priority.

Impact: This topic supports Chemical and Biological Defense Program goals by developing therapeutic medical countermeasures against members of the Filoviridae family of viruses. Outcomes from these studies are intended to provide options for pre-Emergency Use Authorization (EUA) candidates for regulatory review and potential for interim fielding for limited, defined populations in the event of a declared emergency. This MCM will be used to treat the Warfighter following unintentional or natural exposures to these viruses.

Responsive Proposals may address:

Broad-spectrum therapeutic candidates are preferred but not required.

Medical devices including hemofiltration and viral ligand binding devices will be considered.

Drug classes may include viral replication inhibitors, blockers of viral uptake, translocation, modulators of the host response, enhancement of viral degradation and clearance interruption of cell pathways resulting in viral infection and any other medical countermeasure aimed at increasing efficiency of palliative medicine, reducing mortality and/or morbidity of infected subjects.

The following are not of interest and considered outside of the scope of the topic:

a. Protein, peptide, peptidomimetic based therapies.
b. Phage-based therapies.
c. Basic research studies focusing on host-pathogen interaction including target identification and/or validation or structural analysis of antiviral targets.
d. Efforts directed toward the reformulation of FDA approved, late-development, or failed therapeutic candidates for BWA or other indications.

Efforts will be prioritized according to preliminary data in order of decreasing priority:

1. Preliminary Data

    a. Proposals with extensive preliminary data demonstrating a correlate of efficacy *in vitro* and *in vivo* with optimized assays and conditions in place to develop and characterize PD and PK, cytotoxicity, ADME and viral inhibition and clearance for any combination of pathogens.

b. Proposals with limited preliminary data demonstrating limited correlative efficacy *in vitro* and *in vivo* with limited optimized assays and conditions in place to develop and characterize PD and PK, cytotoxicity, ADME and viral inhibition and clearance for any combination of pathogen/s.

c. Proposals with only *in vitro* data demonstrating potential therapeutic efficacy. Proposals without *in vitro* preliminary data, but with similar data and/or validated approaches in other models or systems.

Priority will be given to submissions which provide Proof of Concept (POC) and Proof of Principle (POP) data from validated small and large animal models of filovirus induced disease. Submissions should include scope of work, development paths and regulatory strategy and may encompass both research and development domains of research. Translational science indicating the safety and potential for disease-modifying effects of potential candidates should outline the basis for the submission.
Offerors are encouraged to develop R&D collaborations with other organizations in Government, academia, and the private sector to broaden and strengthen their capabilities. Where possible, Offerors are encouraged to take advantage of specialized resources in DoD and other Government agencies such as facilities/capabilities for biocontainment, collections of biothreat pathogens, Core testing, or advanced manufacturing.
DoD has awarded a contract (W911QY-13-C-0010) to establish an Advanced Development and Manufacturing capability (ADM). In addition to providing a BSL-3 capable, multiproduct manufacturing facility for biologic products, the ADM and a consortium of teaming partners can support development of medical countermeasures from discovery through FDA approval. This includes the facilities, equipment and expertise necessary to perform nonclinical, clinical, process development, and regulatory activities. Respondents interested in discussing potential collaborations with the ADM may inquire through the BAA.

Topic: CBM-04
Animal Model Development for Evaluation of Therapeutic Medical Countermeasures.

Objectives: This topic seeks proposals from qualified performers with a track record of developing animal models for evaluating therapeutic drug candidates. Common drawbacks of current models include the lack of longitudinal capabilities using imaging and/or telemetric monitoring, lack of defined challenge dose - response modeling, no translational, predictive biomarkers for trigger-to-treat, assessing disease progression or for monitoring therapeutic efficacy. It is also important to recognize that a major priority for the DoD is aerosol challenge so these models need to show reproducible aerosol delivery of challenge agents over a range of doses. Specifically, we are looking for animal models that address capability gaps in a) alphaviruses, b) filoviruses and c) botulinum toxin.

Virus:

<u>Alphavirus:</u>

GAPS: The DoD is interested in animal models that allow for routine testing of therapeutic drugs against alphaviruses. The most significant gap in current alphavirus animal models is the inability to easily determine brain exposure by the virus; inability to assess blood brain barrier permeability to potential therapeutic; inability to monitor candidate drug levels in the CNS; inability to monitor cytokine or seizures on a longitudinal basis.

Desired Capabilities:

- Longitudinal studies that clearly show the progression of the disease. Initial focus can be on Venezuelan Equine Encephalitis, but Western and Eastern models would be long term interests as well. It is expected that the studies will clearly define the time course for when the virus directly migrates to brain via aerosol challenge by-passing general circulation and/or crosses the blood brain barrier (BBB) as well as infecting other organs and lymph nodes throughout the body. Preference will be given to those technologies that allow for determination of time course and challenge dose-response relationship studies throughout the course of disease progression, such as Positron Emission Tomography (PET) or similar imaging techniques. Longitudinal studies should also be able to monitor drug exposure within the CNS, host response to pathogen and be able to quantify seizure liability and other hemodynamic parameters.
- Defined full dose-response to aerosol exposure (0 – 100% mortality). At minimum, the model must be robust enough to define confidence intervals that will lay the groundwork for effective pharmacological studies.
- Ability to clearly define trigger-to-treat parameters that include pathogens and clinical variables.
- Animal models must be able to be dosed by the same intended route as projected for human use.

<u>Filovirus:</u>
GAPS: The DoD is interested in animal models that allow for routine testing of therapeutic drugs against filoviruses. Since rodent models of filoviruses may not provide sufficient congruency to humans pathogens and disease course better models are needed. Therefore, DoD is interested in animal species that are more closely representative of the human disease, and will be ethically and economically useful for therapeutic drug development. These models must present features of human disease; from onset to terminal stages. The model could include not only traditional infectious signs and symptoms, but novel pathophysiological signs and biomarkers as well. The model will be used for safety and efficacy assessment for development of filovirus therapeutics.

Desired Capabilities:

- Defined full dose-response to aerosol exposure (0 – 100% mortality). At minimum, the model must be robust enough to define confidence intervals that will lay the groundwork for effective pharmacological studies.
- Preference will be given to Marburg virus, though other filoviruses may be considered.
- Develop clear assays that could be acceptable for trigger to treat for eventual pharmacological studies.
- Ability to longitudinally monitor aspects of disease pathophysiology that mimic the human disease counterpart.
- Animal models must be able to be dosed by the same intended route as projected for human use.

Toxins:

Botulinum toxin (BoNT)

GAPS: To better develop therapeutic MCMs against BoNT and evaluate toxic disposition, improved animal models are being sought. The priority is serotype A, though other serotypes may also be desirable. The extreme toxicity of botulinum serotype A toxin has hindered the development of sophisticated animal models; The inability to reproducibly challenge with a non-lethal dose complicates development of MCMs; A further limitation with current models is the inability to non-invasively monitor, the animal over the course of disease progression.

Desired Capabilities:

- Defined full dose-response to aerosol exposure (0 – 100% mortality). At minimum, the model must be robust enough to define confidence intervals that will lay the groundwork for effective pharmacological studies.
- Telemetric monitoring of the animal, especially with regard to cardiac and pulmonary function. Priority will be given to existing successful animal models in which telemetry monitoring systems have been established.
- Chronic consequences of marked by chronic neurologic deficits such as musculo-skeletal and respiratory consequences will be most appreciated
- Animal models must be able to be dosed by the same intended route as projected for human use.

Offeror's are encouraged to develop academic, government or contract research organizations (CRO) collaborations as necessary, especially if access to required BioSafety Level laboratories is necessary.

Topic: CBMV-01
Investigation of Next-Generation Nucleic Acid Vaccine Platforms

Background: Despite the many significant strides toward the development of rapid production platform technologies that underpin a broad capability for pretreatment medical countermeasure responses to emerging and unanticipated threat agents, the translation of laboratory-tested concepts to feasible technologies continues to require incremental improvements. Platform technologies that fall into this nexus ideally avoid components that invoke preexisting immunity that subsequently interfere with applications of the platform when targeting a secondary indication. Nucleic acid vaccines fall into this category, in that nucleic acids have been shown to function effectively through delivery of antigen alone. Still, a current mechanism for effective nucleic acid vaccine delivery with proven feasibility in animal models requires the use of bacterial master- and working-seed banks, as was well as an electroporation device for delivery, which increases the logistical footprint of this promising technology. It is desirable, therefore, to identify strategies that further minimize the technological requirements for effective application of nucleic acid pretreatments, thereby reducing risk and shortening the production timeline for vaccine conception through vaccine release for clinical use.

Impact: This topic supports the Chemical and Biological Defense Program's goals by investigating next-generation rapid platforms for production of pretreatments to be used as biodefense vaccines. This work will test feasibility next-generation rapid production platforms for pretreatments that ultimately could be used to protect the Warfighter from intentional to biological threat agents.

Objectives: The objective of this topic is to:
Offerors shall use a mature DTRA-funded VEE DNA vaccine (http://www.ncbi.nlm.nih.gov/pubmed/21450977) candidate, which will be provided as Government-Furnished material (GFM), as the baseline to assess the feasibility of promising next-generation nucleic acid platforms and nucleic acid formulations for rapid production of effective pretreatments.
Preference will be given to proposals employing a nucleic acid as the core platform technology using formulations that significantly improve the immunogenicity of the VEE virus immunogen compared to the baseline GFM:

- Using defined strategies that incrementally improve outcomes, such as but not limited to enhanced immunogen expression, improved cross-presentation, increased involvement and recruitment of antigen presenting cells (APCs), and/or enhanced APC stimulatory functions and antigen uptake;

and/or

- Incorporating one or more siRNA expression elements to achieve incremental improvements in DNA vaccine immunogenicity.

and/or

- Resulting in but not limited to focused immunodominance, rapid onset of immunity, reduced dose requirements and/or improved immunological memory for durable immunity.

In addition, proposals should include a plan to evaluate the vaccine candidate(s) in a small animal model, preferably guinea pigs. Priority will be given to those proposals that aim to induce a rapid onset of immunity, ideally by 28 and no later than 90 days. Priority will be given

to those proposals that aim to induce durable immunity greater than 1 year post the primary series using the minimum number of doses, wherein a single dose is preferred but the primary series should not be more than 3 doses.
Deliverables will include:

- A technical data package describing the methods, study results, compositions, formulations and other requirements that are drivers of enhancements in the next-generation DNA vaccine.
- A straw-man proposal as to how the next-generation DNA vaccine platform will be utilized, including logistics, producibility and regulatory metrics, by the DoD to provide a means for rapid, flexible and agile manufacturing of MCMs to meet emergency and urgent Warfighter needs.
- A non-exclusive license for DoD to use the Offeror's next-generation DNA vaccine technology for any purpose in any government laboratory it designates.

ADM Utilization: The DoD has awarded a contract (W911QY-13-C-0010) to establish an Advanced Development and Manufacturing capability (ADM). In addition to providing a BSL-3 capable, multiproduct manufacturing facility for biologic products, the ADM and a consortium of teaming partners can support development of medical countermeasures from discovery through FDA approval. This includes the facilities, equipment and expertise necessary to perform nonclinical, clinical, process development, and regulatory activities. Respondents interested in discussing potential collaborations with the ADM may inquire through the BAA. Nota bene: The decision to, or NOT to, use the ADM is totally independent of, NOT a criterion for, and will have NO bearing on the decision to select a proposal for funding.

Topic: CBMV-02
Medical Countermeasures for Western, Eastern and Venezuelan Encephalitis Virus:
Mucosal Vaccine Development and Identification of Markers of Infection in Animal Models

Background: The encephalitic alphaviruses, Western (WEEV), Eastern (EEEV), and Venezuelan (VEEV) Equine Encephalitis Viruses, are able to cause severe disease in humans via either natural, mosquito-borne infection or accidental laboratory exposure. The illnesses associated with these viruses can result in severe neurological symptoms and mortality ranging from ~1% (for VEEV) to as high as 70% (for EEEV). Currently there is no licensed vaccine against WEEV, EEEV and VEEV, and standardized markers of infection in animal models of WEEV, EEEV and VEEV are lacking. The goal of this Broad Agency Announcement is to solicit proposals aimed either 1) at developing mucosal vaccines against WEEV, EEEV, and VEEV, or 2) at identifying reliable indicators of infection that can be applied to FDA approval of medical countermeasures.

Impact: This topic supports the Chemical and Biological Defense Program's goals by providing 1)candidate mucosal vaccine(s) against Western, Eastern and Venezuelan equine encephalitis virus, and 2) reliable markers of infection an WEEV, EEEV, and VEEV animal models. These

two topics, in turn, protect the warfighter against Western, Eastern and Venezuelan equine encephalitis viruses and enable future licensure of vaccine candidates.

Objectives:
Prioritization: In their proposals applicants should address the encephalitic alphaviruses according to the following order of priority: VEEV (highest priority), followed by EEEV and then WEEV (lowest). Applicants should structure their proposals accordingly, with priority tasks within the Base Period and optional tasks in following Option Periods.
Subtopics: Applicants may respond to either Subtopic A. Mucosal Vaccine Development or Subtopic B. Reliable Markers of Infection in Animal Models. It is not necessary or desired by DTRA that both subtopics be covered in the same proposal.

A. Mucosal Vaccine Development

The objective of this portion of the topic is to solicit proposals for vaccine development that will protect against Western, Eastern and Venezuelan equine encephalitis viruses and that can be delivered via mucosal routes, including intranasal, sublingual, or buccal. Proposals that address the following will be given consideration:

- Vaccine candidate(s) formulated for intranasal, sublingual or buccal administration.
- Demonstration of immunogenicity:
  i. The project should fully characterize the immune response after vaccination and challenge. Analyses may include but are not limited to antibody and T cells responses, with a focus on both quantitative and qualitative analyses such as epitope mapping and T cell repertoire characterization.
  ii. The proposal should include a plan to determine the mechanism(s) of protection.
- Demonstration of efficacy:
  i. The project should evaluate vaccine candidate(s) in small-intermediate animal models challenged via aerosol. Priority will be given to those proposals which also include efficacy studies in an outbred and inbred animal model.
  ii. The proposal should include a plan to evaluate efficacy in a nonhuman primate challenged via aerosol.
- Proposals should aim for focused immunodominance, rapid onset of immunity, reduced dose requirements, and immunological memory for durability.
- Applicants should describe how their vaccine candidates would be manufactured and provide at least proof-of-concept data to demonstrate manufacturability and the feasibility for scale up.
- Candidates that demonstrate potential compatibility with military operations (CONOPS) will be given priority. Factors will include ease of administration without specialized medical devices, minimal or no cold-chain requirement,

storage stability, and minimal number of administrations to generate protective immunity.

B. Reliable Markers of Infection in Animal Models

DTRA would like to move away from traditional animal models that depend on a lethal outcome to test for vaccine efficacy. The objective of this subtopic is to solicit proposals for the development of reliable alternative markers of alphavirus infection. These markers are needed to guide discovery and development of effective vaccines and other medical countermeasures in animal models.

Applicants should address the following attributes of their proposed markers:

- They should be reliable, quantitative indicators that can be rapidly evaluated.
- They should be easily applied without harm to the animal and not be overly invasive.
- Markers should be usable in real time or as close to real time as possible.
- The anticipated costs of marker identification, including any needed supplies or reagents should be reasonable.
- Marker identification should be readily transferable to multiple laboratories and must be determinable under BSL-3 conditions.

Proposals should provide a plan for development of markers of infection that provides a pathway for GLP validation of the marker. The goal should be that the validation data could be filed with the FDA to support approval of alphavirus vaccines or other medical countermeasures. The research to prepare for validation does not itself need to be GLP compliant. Proposals that provide proof-of-concept data and that are farther along the developmental pathway towards validation will receive priority.

Topic: CBMV-03
Vaccines Directed Against *Burkholderia* Species

Background: *Burkholderia mallei* and *Burkholderia pseudomallei* are Gram-negative bacteria that cause glanders and melioidosis, respectively. While infection can occur via multiple routes (ingestion, percutaneous inoculation, and inhalation), the inhalational route is associated with high mortality. Both *B. mallei* and *B. pseudomallei* are considered to be organisms of high risk for deliberate misuse primarily due to the potential for humans to develop disease following inhalational exposure to low bacterial numbers. Successful treatment is complicated by the intrinsic resistance of *Burkholderia* to multiple antibiotics which often results in recurrent disease. Currently there is no vaccine for the prevention of either disease. In addition, *B. pseudomallei* is endemic in the soil and water of many tropical areas across Southeast Asia, the Indian subcontinent, northern Australia, and parts of Africa, South America, and the Caribbean,

adding to the ease with which bacterial strains can be isolated. Due to this concern, combined with the potential public health threat posed to Warfighters in endemic regions, there is an urgent need to develop medical countermeasures (MCMs) for the prevention of melioidosis and glanders.

Impact: This topic supports Chemical and Biological Defense Program goals by providing vaccine candidates for the prevention of melioidosis and/or glanders due to intentional and/or natural exposures to *B. pseudomallei* and/or *B. mallei*. Work under this topic may also generate fundamental information regarding protective immunity against *B. pseudomallei* and *B. mallei*.
Objectives: The objective of this topic is to solicit proposals for melioidosis and/or glanders vaccines that show sufficient efficacy and acceptable safety profiles in relevant animal models. Priority will be placed on vaccines that seek to demonstrate utility against aerosol exposure to both *B. pseudomallei* and *B. mallei*, and include any of challenge agents below:

- *Burkholderia pseudomallei* – MSHR5855
- *Burkholderia pseudomallei* – HPUB10134a
- *Burkholderia pseudomallei* – K96243
- *Burkholderia mallei* – 23344 FMH

Key objectives and decision points should include:

- Demonstration of immunogenicity:
    a. Characterization of the immune response. Analyses may include antibody and T cell responses, with focus on both quantitative and qualitative analyses such as epitope mapping and T cell repertoire characterization.
    b. Determination of mechanisms of protection. Utilization of microbiological and immunological tools to define the mechanism by which vaccine candidate(s) protect.
- Demonstration of efficacy:
    a. The proposal should include a plan to evaluate the vaccine candidate(s) in small animal models: C57BL/6 mice are preferred for initial efficacy studies. Priority will be given to those proposals which also include efficacy studies in an outbred mouse model.
        i. Demonstration of protective efficacy. Protective efficacy may be defined as >80% survival over 30 days and >50% survival over 60 days OR extension of therapeutic window by >28 days. (NOTE: if the latter is considered as the metric of choice, parallel evaluation of the candidate vaccine + therapeutic should be assessed).
        ii. Bacterial load and/or pathology should be assessed in any survivors or euthanized animals.

    b. The proposal should include a plan to evaluate vaccine efficacy in a nonhuman primate model (NHP). Currently, both African Green Monkeys and Rhesus

Macaques are being considered as nonhuman primate models of *B. pseudomallei* infection. African Green Monkeys are the preferred model for *B. mallei* infection.

i. Demonstration of protective efficacy. Protective efficacy may be defined as >80% survival over 45 days and >50% survival over 60 days OR extension of therapeutic window by >28 days. (NOTE: if the latter is considered as the metric of choice, parallel evaluation of the candidate vaccine + therapeutic should be assessed).
ii. Bacterial load/pathology should be assessed in any survivors or euthanized animals.

- Onset to immunity between 28 and 90 days
- Duration of immunity > 1 year
- Logistics:
    a. Minimal number of doses required: Single dose preferred, no more than 3 doses
    b. Limited BSL-3 requirements for production

The following objectives will depend on the maturity of the vaccine candidates being proposed:

- Evaluation of key preclinical toxicity studies. At this stage, it is recommended that engagement with the FDA be pursued in order to assess study design.
- Submission of IND to the FDA (if achievable).

Topic: CBMV-04
Broad Spectrum Prophylaxis of Biological Toxins

Background: The Department of Defense (DoD) is concerned with the threat to the armed services posed by a wide range of biological toxins (biotoxins). Based on historical threats, DoD has been funding priority development of medical countermeasures for three biotoxins, Ricin, *Clostridium botulinum* neurotoxin (BOT) and *Staphylococcus* type B enterotoxin (SEB). However, there has been little or no work to develop MCMs for a diversity of other toxin categories that could in the future pose a threat. These biotoxins include those derived from plants, fungi, marine microorganisms, and bacteria. Their mechanisms of action also are diverse, ranging from blockage of specific molecular reactions or binding to specific receptors, to those acting at multiple sites. Various biotoxins can cause organ-specific morbidity, such as hepatotoxicity, nephrotoxicity or neurotoxicity, or simultaneous failure of multiple organs. Current broad-spectrum treatment options are limited in nearly all cases to supportive and palliative care and developing specific MCMs for all classes of potential bio-toxin threats would be prohibitively expensive.

Therefore, DoD wishes to fund development of broad-spectrum platform technologies that have the ability to provide effective pretreatment or prophylaxis for the widest range of biotoxins. Although the challenge of achieving this objective may appear to be daunting, recent advances

suggest that it may be possible to engineer biomimetic molecules or nanoparticles as MCMs that can serve this protective function. These biotoxin MCMs might, for example, absorb, detoxify or block biotoxins from entering human target cells and organs, or activate the body's natural protective physiological systems.

Impact: This topic supports Chemical and Biological Defense Program goals by developing prophylactic MCMs applicable to a broad range of biotoxins of diverse taxonomic origins. Examples of these toxins include, but are not limited to: fungal toxins (e.g. mycotoxins and amatoxins), marine toxins (e.g. tetrodotoxin, saxitoxins, domoic acid, lipophilic toxins, microcystins and conotoxins), plant toxins (e.g. abrin and other non-ricin toxalbumins) and bacterial toxins (e.g. *C. perfringens* epsilon toxin, shiga-toxin and shiga-like toxins). This solicitation does not cover ricin, SEB and *C. botulinum* toxins, which are covered by other MCM R&D programs.

Objectives: The objective of this topic is to solicit proposals for development of medical countermeasures with the following characteristics:

- The medical countermeasures should provide safe and effective pretreatment or prophylaxis against a range of biotoxins that may pose a threat to the warfighter. The range of biotoxins should be as broad as is reasonable, but it is recognized that it is improbable that a single MCM will provide universal protection.
- However, technologies may be proposed that address a specific molecular or systemic target, provided the model is relevant to several classes of biotoxins that attack that target. Toxin class diversity may refer to either molecular structures or site of action.
- Platform technologies that are adaptable to counter a range of threats are preferred over those which are not adaptable.
- MCMs must have potential to protect against damage to all organs and systems that are targets of the primary or secondary effects of a given biotoxin.
- MCMs that have characteristics consistent with military operations (CONOPS) are preferred to those which are not adaptable to military use. Example of relevant factors include: pharmaceutical stability and avoidance of a cold chain, ease of self-administration by warfighters themselves, long duration of protection, and post-exposure prophylactic efficacy without reduction in the warfighters' performance. Medical devices not commonly found in pharmacies or dispensaries should not be required for administration. No monitoring of blood levels should be necessary.
- Anti-toxin prophylactic MCMs that are in more advanced stages of pharmaceutical development are preferred. At minimum, convincing proof-of-concept data should already be in hand, and the MCMs should be ready for non-GLP toxicology studies.

ADM Utilization: The DoD has awarded a contract (W911QY-13-C-0010) to establish an Advanced Development and Manufacturing capability (ADM). In addition to providing a BSL-3 capable, multiproduct manufacturing facility for biologic products, the ADM and a consortium of teaming partners can support development of medical countermeasures from discovery through FDA approval. This includes the facilities, equipment and expertise necessary to

perform nonclinical, clinical, process development, and regulatory activities. Respondents interested in discussing potential collaborations with the ADM may inquire through the BAA. Nota bene: The decision to, or NOT to, use the ADM is totally independent of, NOT a criterion for, and will have NO bearing on the decision to select a proposal for funding.

Topic: CBMV-05
Development of an Animal Model with Pre-Existing Immunity to *Coxiella Burnetii*

Background: *Coxiella burnetii*, an obligate intracellular bacterium, is the etiologic agent of Q fever, an acute febrile disease that can progress to become a serious chronic illness that results in inflammation of the liver, lung, heart and brain. *C. burnetii* is readily transmitted between hosts and environmental reservoirs, with human infection primarily occurring via the inhalation of infectious aerosols. Currently, a formalin-inactivated vaccine (Q-VAX) is licensed in Australia; however, safety and utilization constraints render it unsuitable for US Warfighters. This vaccine provides near-complete protection in humans, however serious side effects have been observed in individuals either previously exposed to the pathogen or previously vaccinated. Common side effects include tenderness, erythema and oedema at the injection site, and transient headaches. Uncommon reactions can include immune abscesses at the injection site, subcutaneous lumps that have the potential to disperse without intervention, hyperhidrosis, lymphadenopathy, granuloma, myalgia and athralgia[1, 2]. To decrease the incidence of adverse reactions, individuals must undergo a pre-vaccination screening, which consists of two assays, a skin and serological test, that measure different arms of the immune system and past medical history.

While pre-vaccination screenings have significantly lowered the incidence of vaccine-related hypersensitivity, skin and blood tests are time consuming, costly, and may be incorrectly applied or misinterpreted. Most importantly, patient populations with previous exposure to Coxiella are unable to be vaccinated. Therefore, efforts are underway to develop safer Q fever vaccines that will eliminate the requirement for pre-vaccination screening, yet retain vaccine efficacy and safety.

Development and licensure of a Q fever vaccine will proceed under the United States Food and Drug Administration (FDA) Animal Rule. When human clinical trials are not feasible or ethical, the Animal Rule enables licensure of candidate vaccines and therapeutics to proceed when efficacy is demonstrated in well-characterized animal models that reflect human disease. Therefore, suitable animal models with pre-exposure to *C. burnetii* will be required for the development and licensure of candidate vaccines. While the Hartley guinea pig model, which displays common adverse reactions such as the formation of sterile abscesses and granulomas at the inoculation site[3], is reliable, it does not recapitulate the uncommon pathologies associated with vaccination of humans who are already sensitized to Q fever antigens and who may therefore experience serious hypersensitivity reactions if vaccinated. This Broad Agency Announcement seeks to solicit proposals aimed to develop a standardized animal model for assessing common and uncommon vaccine-related adverse reactions that are similar to those observed in humans. This sensitized animal model will enable identification, development and subsequent licensure of Q fever vaccines that do not cause adverse reactions in humans.

Impact: This topic supports Chemical and Biological Defense Program goals by providing a suitable animal model for efficacy and safety testing of Q fever vaccine candidates and subsequent licensure under the FDA Animal Rule.

Objectives: This Broad Agency Announcement seeks to solicit proposals focused on development and standardization of an animal model for assessing vaccine-related adverse reactions that are similar to those observed in humans. Consideration will be given to proposals that include the following:

- Models should be directly applicable to the discovery, evaluation and development of vaccines against Q fever.
- Animals should be sensitized either by pre-immunization followed by *C. burnetii* aerosol challenge, or via aerosol exposure to *C. burnetii* only. Proposals that sensitize animals through other routes, such as by i.p. inoculation of *C. burnetii* will be given lower priority.
- Characterization of the immune response and pathogenesis following sensitization should be performed.
- Following sensitization, models should display the adverse reactions shown below. Characterization of the immune response and pathogenesis should also be established at this stage.
    - Common Adverse Reactions: fever, joint swelling, injection site inflammation, induration, and oedema;
    - Uncommon Adverse Reactions: endocarditis, systemic manifestations such as lymphadenopathy, hyperhidrosis, abscess formation, and granuloma.
- Models should be planned to have sufficient statistical power to make down-selection decisions on vaccine candidates at a reasonable cost.
- Models should use commonly available laboratory animals. In certain cases, specialized breeds may be advantageous and their use is not discouraged provided they are readily available.
- Models that are less burdensome on the time of investigators and facilities will be preferred over those more burdensome. Pain and suffering must be minimized, and all models must conform to animal welfare provisions of their respective institutions, the DoD, the USDA and all applicable laws.

Regulatory Compliance: It is anticipated that a new Q Fever vaccine will need to be approved by the US Food and Drug Administration (FDA) under the Animal Rule. The Animal Rule provides a pathway for FDA approval of a new vaccine (21 CFR 19 601.90) in the event that human clinical trials are not feasible or ethical. The Animal Rule enables efficacy to be demonstrated in well-characterized animal models that reflect human disease. ***Therefore, the model development to be funded under this Topic must be designed from the start for eventual compliance with the Animal Rule.*** FDA recently updated their relevant Draft Guidance, *Product Development Under the Animal Rule* (see references).

FDA-regulated studies subject to the Animal Rule submitted for approval of a specific therapeutic or vaccine must be conducted in accordance with preexisting requirements under the Good Laboratory Practices (GLP) regulations (21 CFR part 58). This GLP requirement does not apply to the research done to develop an animal model to comply with the Animal Rule, but in developing such models the steps necessary for GLP compliance must be anticipated and executed. In particular, the model will require validation. These requirements are discussed in the applicable FDA guidances. Additional information on FDA guidances is available on FDA's Web site. In addition, FDA guidances related to medical countermeasures for chemical, biological, radiological, and nuclear (CBRN) agents can be accessed through FDA's Medical Countermeasures initiative (MCMi) Web site.

References:

1. Marmion BP, Ormsbee RA, Kyrkou M, Worswick DA, Izzo AA, Esterman A, Feery B, Shapiro RA. Vaccine prophylaxis of abattoir-associated Q fever: eight years' experience in Australian abattoirs. Epidemiol Infect. 1990 Apr; 104(2):275-87.
2. Q-VAX Product Information. http://www.csl.com.au/docs/39/836/Q-Vax_PI_V4_TGA-Approved-17%20January%202014.pdf.
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ADVANCED AND EMERGING THREAT DIVISION (CBS)

TOPIC AREA FUNDING OPPORTUNITIES: Chemical Medical Countermeasures (MCMs)

Topic: **CBS-01**
Organophosphorus Nerve Agent Medical Countermeasures

Goal: Proposals are sought for improved and/or enhanced prophylaxis or therapeutics MCMs to augment the standard treatment regimen for chemical warfare agents (CWA) intoxication. The aim of this optimization is increased survival, reduced morbidity, and decreased neurological adverse effects for emergency use/acute treatment of CWA intoxication.

Background: Present research efforts suggest that the current regimen of countermeasures for the treatment of acute CWA intoxication offer inadequate broad-spectrum protection against multiple and diverse organophosphorus nerve agents (OPNA). Moreover, the standard regimen does not sufficiently protect the Central Nervous System (CNS) function from OPNA-induced overstimulation and neurotoxicity.

Objectives: This topic seeks innovative milestone-driven proposals focused on discovery and early preclinical development of prophylactic or therapeutic MCM candidates that address either the direct neutralization of a broad-spectrum of OPNAs in the blood prior to reaching their target or treatments that provide direct CNS protection. Desirable, but not exclusive, treatments include traditional and non-traditional MCM approaches that function as enhanced circulatory bioscavengers, reactivators of OPNA-inhibited acetylcholinesterase (AChE), anticholinergics, anticonvulsants, or neuroprotectants against OPNA intoxication. Approaches must utilize pharmaceutical industrial best practices driven by iterative cycles between MCM synthesis and *in vitro* evaluation for desired activity and the maintenance of desired drug-like properties prior to *in vivo* efficacy studies.

I. Proposals seeking novel and improved MCM approaches for OPNA intoxication are highly encouraged. Proposals shall clearly outline research to collect early preclinical data supportive of a future MCM's Target Product Profile (TPP) for regulatory submission that demonstrates broad-spectrum protection against multiple, diverse OPNAs or address the aforementioned inadequacies of the current standard MCM regimen. As an example, early preclinical data applicable to either small molecule-based or protein-based MCM material solutions with the following attributes shall be acquired, as appropriate:

a) A broad-spectrum reactivation profile for appropriate animal and human acetylcholinesterase (AChE) inhibited by G-type OPNA (e.g. represented by GA, GB and GF) and V-type OPNA (e.g. represented by VX and VR).
b) Stability in appropriate animal model, human plasma and microsomes.
c) Permeability in MDR1/MDCK cell assays predictive of CNS accessibility prior to selection for *in vivo* analysis.
d) Pharmacokinetics (PK) and brain to plasma partitioning supporting a fast onset of action with adequate CNS drug exposure levels as determined in unchallenged guinea pigs.

e) Statistically relevant and appropriate safety profile in appropriate animal models as demonstrated by the therapeutic index, defined by dividing the drug plasma levels at the maximum tolerated dose by the minimal efficacious plasma drug concentrations.
f) Statistically supported evidence of *in vivo* efficacy of small molecule MCMs delivered by intramuscular (IM) injection in a clinically acceptable formulation in animal models subcutaneously exposed to OPNA (or Paraoxone). The MCM candidates shall increase survival or decrease major morbidity (incidence of motor convulsions or other CNS dysfunction) compared to the current antidote regimen in appropriate animal efficacy models with s.c. challenge by the OPNA (or Paraoxone). Efficacy models (without telemetry implants) shall include 1) AChE reactivation in tissues (brain homogenate, blood, heart skeletal muscle, kidney and diaphragm); 2) ability to increase survival; and 3) ability to reduce morbidity (incidence of motor convulsions or other CNS dysfunction).
g) Bioscavenger MCMs shall demonstrate adequate catalytic efficiency (i.e. k_{cat}/K_M) $\geq$ $x10^7$ $M^{-1}min^{-1}$.
h) Targeted MCMs approaches shall demonstrate *in vivo* protective ratio $\geq 2LD_{50}$ for G-type and V-type OPNAs (or $4LD_{50}$ for Paraoxone)
i) Bioscavenger MCMs should demonstrate circulatory profiles at $T_{max} \leq$ 24 hours; protective ratio maintained $\geq$ 10 days.
j) Targeted MCM approaches shall demonstrate acceptable immunogenicity and safety profiles in an appropriate animal model.
k) MCMs approaches shall demonstrate additional Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) data as applicable. Applicants are responsible for acquiring ADMET data.

II. For the purpose of this effort, priority review will be given toward the following:

1) Proposals with extensive preliminary data (well established structure-activity relationship of advanced hit/lead chemical series, *in vitro* assessment and validation of hits/leads, ADMET/PK, proof-of-concept *in vivo* efficacy, acute toxicity, and defined mechanism of action) as MCMs for OPNA treatment will be given highest priority. MCM candidates must clearly demonstrate the potential for activity against OPNA and/or appropriate surrogates.

2) Proposals with MCMs in advanced stages of development either through prior efforts, or in conjunction with other ongoing, complementary work outside this topic, involving hit-to-lead optimization, lead validation and early preclinical (non-GLP) efforts.

3) Efforts directed toward the reformulation of FDA approved, late-development, or failed therapeutic candidates with overlapping or similar indications for OPNA intoxication or their associated/secondary indications are welcomed. Additionally, this may include the use of Design of Experiments (DOE) methodology to assess the optimal outcome of combining one or more candidate MCMs, for example, with varying combinations of the current regimen of countermeasures for the treatment of acute OPNA intoxication regimen-which includes:

a) pre-exposure administration of Pyridostigmine Bromide (PB);
b) post-exposure administration of atropine;
c) post-exposure administration of Pralidoxime Chloride (2-PAM); and
d) post-exposure, post-symptomatic administration of Diazepam-to afford enhanced protection against a broad-spectrum of OPNAs.

III. The following is NOT of interest and considered outside of the scope of the topic: basic research studies focusing mainly on target validation, identification of activities, computational studies or confirmation of hits without demonstration of dependencies and timing associated with a go/no-go decision point in the base year to support drug-like compounds development for medical countermeasures against OPNAs.

IV. Offerors are encouraged to develop R&D collaborations with other organizations in Government, academia, and the private sector to broaden and strengthen their knowledge, experience and capabilities. Additionally, offerors are encouraged to take advantage of specialized resources in DoD and other Government agencies such as facilities/capabilities for chemical surety certification, ADMET testing, or advanced manufacturing.

TOPIC AREA FUNDING OPPORTUNITIES: Threat Agent Science (TAS)

Topic: CBS-02
Correlate Bacterial Degradation on Surfaces, in Response to Environmental Conditions, with Bacterial Degradation in Aerosols for the Purposes of Validating a Predictive Model.

Background: The goal of this topic is to establish a validated model that will estimate viability or degradation of an aerosolized biological warfare agent subject to operationally relevant, environmental conditions based on complementary surface studies. According to Stuart and Wilkening [1] our current understanding of environmental degradation of a biological agent does not provide an accurate hazard assessment and therefore cannot inform appropriate emergency response measures. Current aerosol studies provide data to inform hazard models; however, such studies are high risk to the performers and are lengthy and costly when compared to surface studies. Additional research on degradation rates for spore and vegetative bacteria clusters would provide more accurate inputs to the hazard prediction model. Current threat assessment and hazard prediction models do not necessarily take into consideration the shielding effect by the bacteria cluster to the interior cells. Studies suggest that the clusters provide interior spores protection from UV radiation and enhance survivability of the spores, impacting the overall degradation rate needed to predict the hazard [2, 3]. The development of an experimentally validated model that allows for a surface study of environmental exposures to be scaled to aerosol exposure via a correlation factor would enable the estimation of degradation rates using literature data and timelier and safer experiments. Such a model would help provide the more accurate decay rates that include cluster shielding to inform the hazard prediction models.

Impact: A validated model for estimating the viability or degradation of an aerosolized biological warfare agent from complementary surface studies would provide the means to

enhance current aerosol hazard risk assessment models. Also, using surface studies instead of aerosol studies to predict the aerosol hazard will enable more experiments to be conducted safely and at a lower cost. The model would inform aerosol hazard prediction models for emergency responders.

Objective:

- Establish or expand a currently available model that estimates the viability or degradation of an aerosolized biological warfare agent subjected to operationally relevant, environmental conditions from surface studies.
- Model should be experimentally validated and be able to scale surface study experiments on different particle sizes.
- Verification of the model could involve a set of experiments testing various environmental factors (e.g. UV fluence) with various media preparations of spore and/or vegetative bacteria.
- Model could also take into consideration the shielding effect by the exterior of clusters of spores and vegetative bacteria since recent research indicates the importance of this affect [2, 3]

References:

1. A. L. Stuart and D.A. Wilkening. 2005. Degradation of biological weapons agents in the environment: implications for terrorism response. Envir. Sci. Technol. 39(8): 2736-2743.
2. J. Kesavan, Schepers, D. Bottiger, J and Edmonds, J. 2014. UV-C Decontamination of aerosolized and surface bound single spores and bioclusters. Aerosol Sci Tech. 48: 450-457.
3. F. A. Handler and Edmonds, J. M. 2015. Quantitative analysis of effects of UV exposure and spore cluster size on deposition and inhalation hazard of *Bacillus* spores. Accepted for publication.

Topic: CBS-03
Method Development to Quantitatively Determine Microbial Damage

Background: The goal of this topic is to promote the development of methods and data that would transition to assays and operating procedure to measure damage, physical and genetic, to viable bacteria as a result of exposure to stress (e.g. shear/mechanical, environmental exposure, decontamination). Current methods to evaluate inactivation or viability of agent exposed to stress typically use media culturing of bacteria. However, exposure to stresses likely results in damage to the bacteria that may affect phenotypic properties (e.g. persistence or virulence) but which does not impact its ability to grow on media, but may impact its ability to infect a host. There is also the possibility that non-culturable cells might still be viable and could recover under the correct conditions. The development of methods to detect the type of cell damage (e.g. membrane permeability), and degree of damage and correlate this to infectivity could provide an additional quantitative method for assessing whether or not the damaged bacteria could pose a threat.

There are a number of existing methods that potentially could be used and/or refined including but not limited to qPCR, fluorescent indicators, scanning and other microscopy techniques, and nucleic and immune assays. qPCR has successfully correlated to cell viability for some stress exposure such as isopropyl alcohol [1] but is limited to when the stress has caused cell damage. Additionally, nucleic acid-based assays and immunoassays for the detection of Bacillus spores can be affected by the type of stress experienced by the bacteria [2]. Fluorescent indicator dyes are potentially useful since they provide rapid detection of cell viability and injury due to stress but depend on whether damage to the membrane has occurred [3, 4]. Methods dependent on cell membrane damage would miss stress induced by UV light since it causes DNA damage but not necessarily membrane damage [4]. Further method development is needed to determine type and quantity of damage caused by various bacterial stresses.
Impact: The development of methods that assess the overall and amount of damage, physical and genetic, inflicted to viable bacteria from various stresses would inform assay development and methods to verify sterility of a sample.

Objective:

- Create methods that quantifies the damage inflicted on a cell and bacterial clusters from a given type of stress that would transition into knowledge for assay development or sterility verification methods.
- Proposed bacteria, gram-positive, gram-negative and/or their surrogates, will be of interest to the DoD .
- Methods should include those that assess the degree of damage and assign a quantitative ranking or rating to describe the level/type of cell damage.
- The proposed studies could also include susceptibility of liquid versus dry powder bacterial preparations to gamma radiation.

References:

1. Nocker, A., Cheung, C. Y., Camper. A. K. 2006. Comparison of propidium monoazide with ethidium monoazide for differentiation of live ve. Dead bacteria by selective removal of DNA from dead cells. J. Microbiol. Methods 67: 310-320.

2. Dang, J. L., K. Heroux, J. Kearney, A. Arasteh, M Gostomski, and P. A. Emanuel. 2001. Bacillus spore inactivation methods affect detection assays. App. And Enviro. Micobiol. 67: 3665-3670.

3. Lado, B. H. and A. E. Yousef. 2002. Alternative food-preservation technologies: efficacy and mechanisms. Microbes and Infection 4: 433-440

4. Nocker, A. K. E. Sossa, and A. K. Camper. 2007. Molecular monitoring of disinfection efficacy using propidium monoazide in combination with quantitative PCR. J. of Microbiol. Methods 70: 252-260.

Topic: CBS-04
Environmental Metagenomics to Explore Microbial Community Associations and Seasonal Drift of Ebola and Other DoD-Relevant Pathogens in the AFRICOM AOR.

Background: Microbes do not persist in isolation, but are part of micro- and macrobiotic communities. Viruses that infect bacteria (bacteriophages) are environmentally co-located with their cognate hosts. The same is true of nonbacterial viruses, their hosts and reservoirs, otherwise they would not be able to replicate and evolve over time.

One aspect of viral biology is the ability of some to jump species, and even phyla. The Tobacco Ringspot Virus is an RNA virus that infects tobacco plants, but has recently jumped to honeybees via contaminated pollen. It is a prime suspect in bee colony collapse syndrome.

Other than its apparent origins in the Congo River Basin, virtually nothing is known about the ecology of Ebola, its association with non-human organisms, and the mechanisms by which the currently known variants have arisen. The development of high quality temporal and geospatial metagenomic information on Ebola and other indigenous pathogens (e.g., Lassa fever, Chikungunya, yellow fever, etc.) would provide valuable situational awareness for disease prediction and avoidance.

Objectives: Congo river proof of principle:

- Collect water samples starting from Malebo Pool and selected points upstream within the Congo River main channel.
- Process, sequence and archive water samples and/or libraries.
- Expand survey and analysis to the major tributaries of the Congo River, using information developed in the preceding effort where possible.

Impact: Metagenomic analysis of environmental samples can provide information about fluctuations in biotic content if it is tied to robust temporal, climatic and geospatial data. To the extent that associations among metagenomic signatures are statistically significant, this data could provide indirect indications of the presence of pathogens, even if they are below the limit of analytic detection. The information developed from this effort will also provide a better understanding of microbial associations within complex microbial communities.